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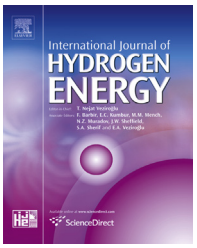

Performance of batch solid state fermentation for hydrogen production using ground wheat residue

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ABSTRACT

Ground wheat (21 g) was subjected to batch solid state dark fermentation for bio-hydrogen production. *Clostridium acetobutylicum* (B-527) was used as the culture of dark fermentation bacteria at mesophilic conditions. Effects of moisture content on the rate and yield of bio-hydrogen formation were investigated. The highest CHF (1222 ml), hydrogen yield (63 ml H_2 g^{-1} starch), formation rate (10.64 ml H_2 g^{-1} starch h^{-1}) and specific hydrogen formation rate (0.28 ml H_2 g^{-1} biomass h^{-1}) were obtained with a moisture content of 80%. Nearly complete starch hydrolysis and glucose fermentation were achieved with more than 80% moisture content and the highest substrate conversion rate (21.9 mg L^{-1} h^{-1}) was obtained with 90% moisture content at batch solid state fermentation producing volatile fatty acids (VFA) and H_2.

Introduction

Bio-hydrogen production from organic wastes is an attractive approach since it facilitates waste treatment beside producing a valuable energy carrier [1,2]. Dark fermentation for bio-hydrogen production from biomass is starting with acid or enzymatic hydrolysis to get a concentrated sugar solution and followed by the acetogenic–anaerobic bacteria pretreated from mixed cultures to produce volatile fatty acids (VFA), hydrogen and CO_2 [2–4]. Many researchers reported that they have used suspended and immobilized cultures with different biomass wastes containing biodegradable carbohydrates such as cheese whey, olive mill wastewater, ground wheat residue, food waste, baker's yeast, corn stalk wastes, sugarcane bagasse etc. which are non-toxic industrial effluents [1,2,5–7]. These kind of reliable carbon sources are inexpensive for hydrogen production and ground wheat residue was used as substrate for bio-hydrogen production in the literature by dark fermentation under mesophilic conditions with different organisms [8–10]. Anaerobic sludge, heat treated anaerobic sludge and pure cultures of *Clostridia* and *Enterobacter* species were used for bio-hydrogen production by dark fermentation [10–17]. For instance ground wheat was subjected to a *Clostridium pasteurianum* and *Clostridium butyricum* mixture in our previous paper showing a hydrogen yield of 109 ml H_2 g^{-1} TS [8]. Also pre-treated anaerobic sludge was used for hydrogen production with acid hydrolyzed wheat under thermophilic conditions giving a maximum cumulative hydrogen gas and hydrogen gas formation of 752 ml and 7.42 ml H_2 h^{-1}, respectively [17].

In the last decades; there is an increasing interest to solid state fermentation having many advantages over submerged fermentation. Solid state fermentation (SSF) is termed as the growth of microorganisms on an adequately moistened non-soluble medium in the absence or near absence of free

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moving water and air [18–24]. In industry this system is utilized to produce desired products in controlled reactors like fine chemicals and value-added products (ethanol, mushrooms, enzymes, amino acids, organic acids, biologically active secondary metabolites, single cell protein, etc.) for its advantages like giving higher yields or better product formation efficiencies with less water requirements over submerged fermentation [19,20,23,25]. The cost of the system is lower due to efficient substrate utilization. Compared to submerged systems; it is a simpler process, reduces energy, have limited operational problems, possible to apply nearly same habitat for some organisms, less cost of initial investment, and gives less liquid waste to be treated [19,23,25]. However there are some problems of SSF like, effective mixture, difficult control of realistic data like pH, heat, humidity, higher impurity product, increasing recovery product costs. Also the absence of water limits the activity of the microorganisms like fungi, yeasts and some kinds of bacteria in SSF [19,23,25].

In the literature there are many researches using SSF for enzyme, organic acids, ethanol, and single cell protein etc. production [26–32]. There are also papers reporting SSF for biogas production [33,34]. However, there are not many studies investigating bio-hydrogen gas production with solid state fermentation. Some researchers used SSF for enzyme production as a pre-step for hydrogen production [30–32]. Han et al. (2016) developed a novel combined bioprocess for hydrogen production where food waste was utilized with solid state fermentation to produce glucoamylase and protease enzymes with *Aspergillus awamori* and *Aspergillus oryzae*. Then the produced enzymes were used to hydrolyze food waste and the residue was subjected to *Biohydrogenbacterium R3* to produce 52.4 ml H_2/g food waste. Vazquez and Varaldo (2009) optimized the total solids (20.9% TS) and alkalinity ratio (0.11 g $CaCO_3$ g^{-1} dry substrate) for hydrogen production with solid state fermentation. They found the highest H_2 productivity and yield as 463.7 N ml kg^{-1} d^{-1} and 54.8 N ml g^{-1} VS_{rem}, respectively, at an alkalinity ratio of 0.25 [35]. Vazquez et al. (2005) evaluated the effects of temperature on hydrogen production in a semi-continuous solid state fermentation media. H_2 production increased with temperature (35 °C–55 °C) and 80% of the maximum yield based on 4 mol H_2/mol hexose was achieved in this study [36]. The same group evaluated the performances of municipal solid wastes and agro-industrial wastes under solid state fermentation. Mesophilic and thermophilic conditions focusing on total gas production were compared in batch and semi-continuous processes where they got more methane gas production but also promising results of hydrogen gas [37–39].

In SSF, water activity is important in the fermentation media, helping diffusion of solutes and gases. If the water activity is not enough the cell metabolism slows down, due to lack of substrates or just the opposite of substrate or product inhibition. Also the transfer of water affects the mechanical structure (plasmic membrane) resulting an unbalanced membrane permeability and transport mechanism. Furthermore; the intracellular or extracellular moisture content effects the maintenance of the functional properties of some enzymes. In solid state fermentation bacteria needs higher water activities than fungi and should be higher than 0.9 [33,40,41]. Therefore, the water content is a key parameter in SSF and the main objective of this study was to investigate the yield and rate of bio-hydrogen production by solid state dark fermentation of ground wheat residue with pure culture *Clostridium acetobutylicum* (B-527) for different moisture contents.

Materials and methods

Experimental set up

310 ml Serum bottles (Isolab-Germany Boro 3.3) were used for batch solid state fermentation experiments. Silicone stoppers, screw caps and metal valves were used to avoid gas leakage from the bottles. The ground wheat used for the experiments contained approximately 97% (ww^{-1}) starch and gluten, 3.4 mg g^{-1} total nitrogen and 1.72 mg g^{-1} phosphate-P. The grinded wheat was prepared by drying at 60 °C to a constant weight and sieved through a 205 μm diameter mesh. Anaerobic conditions were maintained by adding 0.02 (w/v) Na-thioglycolate and passing Argon gas from the head space of the bottles for 5 min at the beginning of the experiments.

The bottles were autoclaved and then incubated in an incubator at 37 °C and mixed several times a day manually. The initial ground wheat residue concentration was 21.1 ± 0.2 g with different moisture contents of 40, 50, 60, 70, 80, 90% (w/w) by considering the water in the inoculums suspended on a wet basis. Water activities were measured in a moisture analyzer showing a range of 0.981–0.988 (MARKA). Therefore all tested experiments were shown in terms of moisture content [33,40,41]. The initial biomass (cell) concentration was constant (37.5 g) in all bottles. The fermentation media contained (w/v) 0.39 K_2HPO_4, 0.28 KH_2PO_4, 0.025 $MgSO_4$, 0.01 $FeSO_4$, 0.2 $(NH_4)2SO_4$. The initial pH was set to 7.3. The pH was measured at the end of the fermentation time which was in a range of 5–4.5 in all bottles. A control bottle was also prepared and showed no production.

Organisms

C. acetobutylicum (B-527) was obtained from USDA National Center for Agricultural Utilization Research, Peoria, IL, USA and was cultivated on a medium containing glucose (5 g L^{-1}), peptone (10 g L^{-1}), yeast extract (5 g L^{-1}), K_2HPO_4 (2 g L^{-1}), meat extract (20 g L^{-1}) and agar (10 g L^{-1}). Argon gas was passed through the cultivation medium before incubation. After one day of cultivation, the cells were centrifuged and re-suspended in distilled water in order to obtain high cell density inoculum culture. Then, they were grown on the experimental media containing 10 g L^{-1} boiled wheat powder, with a media given in Ozmihci and Kargı, (2011) [8] in order to adapt the bacteria to hydrogen production media. After 24 h of cultivation, the cells were centrifuged and used as inoculum.

Analytical methods

The samples for various analyses were taken from the bottles of the day prepared for the designed fermentation period. 100 ml water was added to the samples and were shaken to suspend all ingredients in water. Then samples were

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